PATENT
Docket No. 249.0007 0101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Dominic COSGROVE	Group Art Unit:	1644
Serial No.:	10/698,121	Examiner:	Maher M. Haddad
Filed:	31 October 2003		

For: INDUCIBLE LIGAND FOR α1β1 INTEGRIN AND USES

DECLARATION UNDER 37 C.F.R. §1.132
OF DOMINIC COSGROVE

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

1. I am a named inventor on the above-identified application. I received a Bachelor of Science degree from the University of Nebraska, Lincoln, Nebraska, in 1984 and a Ph.D. degree in Biochemistry from the University of Nebraska Medical Center, Omaha, Nebraska, in 1989. I completed postdoctoral work with Diane Mathis, Ph.D. C.N.R.S. at the Faculte de Medecine, Strasbourg, France, in Molecular Immunology from 1989-1991. Since 1991 I have been employed at Boys Town National Research Hospital, Omaha, Nebraska, where I have been Coordinator of the Gene Expression Laboratory, Department of Genetics since 1991, Director of the Division of Cell and Molecular Biology, National Usher Syndrome Center since 2002, and Director of Basic Research, National Usher Syndrome Center since 2005. I am also Associate Professor, Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, Nebraska, and Associate Professor of Medicine, Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center. My areas of research include the cell

and molecular biology of diseases involving the extracellular matrix, with a focus on hereditary disorders, in particular Alport syndrome and usher syndrome. I have over 50 peer-reviewed publications.

2. I am the sole inventor of the subject matter disclosed and claimed in the above-identified patent application, Serial No. 10/698,121, filed 31 October 2003. I have reviewed the Final Office Action mailed 13 October 2006, and the Advisory Action mailed 20 February 2007, with respect to the above-identified application and make this declaration in support of the patentability of claims 5-8, 10-13, 15, 17, 21, 23, 25, 28, 43-52, 54-59, and 61-71.

3. The shared peptide sequence of the binding site on Collage XIII for α1β1 (GAEGSPGL (SEQ ID NO:1)), as identified by phage biopanning an endothelial cell phage display library, was included in the synthesis of the larger peptide sequence GEKGAEGSPGL, which was conjugated to KLH and used as immunogen for the generation of monoclonal antibodies. Three independent hybridomas, MAB 1, MAB 2, and MAB 3, were selected based on positive ELISA binding to plates coated with the immunizing peptide.

4. Exhibit A shows that monoclonal antibodies MAB 1, MAB 2, and MAB 3, against the binding site on collagen XIII for α1β1 integrin, neutralize adhesion of bone marrow derived monocytes to collagen XIII on embryonic fibroblasts. Cell adhesion experiments were performed using murine embryonic primary fibroblast monolayers as collagen XIII presenting cells. Fibroblasts were chosen as they had previously been shown to constitutively express collagen XIII (Hagg et al., (2001) Matrix Biol 19:727-742; Sund et al. (2001) EMBO J 20:5153-5164), avoiding the complication of having to induce collagen XIII on vascular endothelial cells. Monocytes were obtained from the bone marrow of 129 Sv mice and used directly in cell adhesion experiments. Bone marrow-derived monocytes were labeled with cell tracker dye (Molecular Probes, Eugene, OR)

and layered onto confluent fibroblast monolayers in the presence or absence of either an anti-α1 α1β1 integrin neutralizing antibody, or each of three independently derived monoclonal antibodies reactive with the peptide identified by biopanning as the binding site on collagen XIII for α1β1 integrin (MAB 1, MAB 2, MAB 3). The control antibody was an isotype matched non-reactive monoclonal, which had no effect on cell adhesion. After several washes, the total bound fluorescence was quantified. Four independent experiments were performed in triplicate and analyzed statistically (students t-test with Bonferroni correction). Asterisks indicate significant differences in binding ($p > .001$). The control antibody was an isotype-matched murine monoclonal. All three of the monoclonal antibodies against collagen XIII (MAB 1, MAB 2, and MAB 3) blocked cell adhesion by approximately 80%. A neutralizing monoclonal antibody known to block α1β1 integrin binding to collagen ligands (Mendrick et al., (1995) Lab Invest 72: 367-375; Krieglstein et al., (2002) J Clin Invest 110: 1773-1782) also reduced binding of monocytes to the embryonic fibroblasts by approximately 80%. These data indicate that the α1β1 integrin-positive monocytes are binding to type XIII collagen on the embryonic fibroblasts, and that the monoclonals against the putative binding site for α1β1 integrin on collagen XIII block the interaction.

5. Exhibit B shows that monoclonal antibodies against the binding site on collagen XIII for α1β1 integrin bind to a portion of the vascular endothelial cells in 12-week-old integrin α1-null Alport (DKO) kidneys, but not wild type littermates. MAB 2 was labeled with Alexa 568 and injected into the tail veins of wild-type or DKO mice. Twelve hours later, the kidneys were harvested and cryosections counterstained with FITC-conjugated anti-CD31 antibodies. Alexa 568 immunostaining was not observed in wild type kidney cryosections (panel A), but was abundant in DKO kidney cryosections (panel D). The vascular endothelium is immunopositive for CD31 in both wild type (Panel B) and DKO (Panel E) mice. Panel F shows some, but not all, of the vascular endothelial cells in the DKO kidneys are immunopositive for both MAB 2 and anti-CD31. Importantly, all of the Alexa 568 (MAB 2) immunostaining localizes to the vascular endothelium. Exhibit B

shows that blocking antibodies against collagen XIII selectively bind the renal vascular endothelium in diseased mice, but not to that of age and strain matched controls, and bind to the endothelium in Alport kidneys under the stress of normal blood flow.

6. Exhibit C shows that monocyte migration into the interstitial space occurs in areas where collagen XIII-positive capillaries are abundant. Sections from a twelve week old integrin α1-null Alport mouse kidney were immunostained with either anti-collagen XIII antibody MAB 2 (panel A), Anti-CD11b antibodies (panel B), or both (panel C). Panel C shows that the monocytes (in green) are abundant primarily in areas where the capillaries are immunopositive for collagen XIII (red).

7. Exhibit D shows that treatment of Alport mice with MAB 2, a monoclonal antibody against the binding site on collagen XIII for α1β1 integrin, markedly attenuates the efflux of monocytes into the interstitium of Alport kidneys. Alport mice were given bi-weekly intravenous injections of 25 μg of MAB 2 starting at four weeks of age. Three days prior to sacrifice, mice were injected with Alexa 568-conjugated dextrans. At seven weeks of age, kidneys were harvested and cryosections either visualized directly for newly effluxed monocytes (panels A and C) or immunostained for total monocyte accumulation using anti-CD11b antibodies (panels B and D). Panels A and B show untreated Alport mice. Panels C and D show Alport mice given the MAB 2 injections. The fields shown are representative of ten independent animals. Exhibit D illustrates typical fields of transmigrated monocytes over the three-day monitoring period in Alport mice (panel A) and Alport mice treated with the monoclonal antibody MAB 2 (panel C). Comparing panel A (untreated) with panel C (treated) clearly illustrates a marked reduction in the monocyte efflux when Alport mice are given the collagen XIII blocking antibody MAB 2. This translates to a marked decrease in the total accumulation of interstitial monocytes, as indicated by anti-CD11b immunostaining (Exhibit D, compare panels B and D). Exhibit D shows that antibodies for the binding site on collagen XIII for α1β1 integrin block

transmigration of α1β1 integrin positive peripheral blood monocytes into the tubulointerstitial space of Alport mice.

8. Exhibit E shows that treatment of Alport mice with MAB 2 reduces myofibroblasts and fibrosis in the interstitium of Alport kidneys. Cryosections of either control mice (panels A and B), untreated Alport mice (panels C and D), or Alport mice treated with MAB 2 (panels E and F) were immunostained with antibodies against either smooth muscle actin (SMA, panel A, C, and E) or fibronectin (FN, panels B, D, and F). Both myofibroblast accumulation (SMA immunostaining) and fibrosis (as determined by fibronectin immunostaining) are markedly attenuated in renal cortex of MAB 2 treated mice relative to the untreated mice. Thus, the overall reduction in interstitial monocyte accumulation shown in Exhibit D is associated with a similar reduction in myofibroblasts (Exhibit E, panels A and C), as well as fibronectin accumulation (Exhibit E, panels B and D), illustrating that administration of collagen XIII blocking antibodies results in attenuated progression of interstitial fibrosis in the Alport mouse model.

9. I submit that one of skill in the art would conclude from the results and statements in paragraphs 3-8 that one can, with a reasonable expectation of success, treat a subject having an inflammatory disease or other condition where integrin α1β1-positive interstitial monocyte accumulation is observed by administering to the subject an antibody to Collagen XIII that disrupts the interaction between Collagen XIII and α1β1 integrin (claims 7, 8, 10-12, 28, 54, 61, and 67); reduce selective efflux of integrin α1β1-positive monocytes into the interstitium of chronically inflamed tissues by contacting the α1β1 integrin on peripheral blood monocytes with an antibody to Collagen XIII that interferes with the interaction between Collagen XIII and α1β1 integrin (claims 13, 15, 45, 48, 55, and 62); reduce the rate of monocyte efflux into the interstitial space of chronically inflamed tissues by contacting the tissue with an antibody to Collagen XIII wherein the antibody blocks Collagen XIII from binding with α1β1 integrin (claims 17, 21, 46, 49, 56, 63, and 69); block the interaction of α1β1 integrin on peripheral blood

monocytes with Collagen XIII on vascular endothelium of chronically inflamed tissues by the monocytes, the vascular endothelium, or both with an antibody to Collagen XIII (claims 23, 25, 47, 50, 64, and 70); treat a patient having chronically inflamed kidneys associated with an accumulation of α1β1 integrin positive monocytes in the interstitium by administering to the patient an antibody to Collagen XIII, wherein the antibody reduces the rate of efflux of α1β1 integrin positive monocytes into the renal interstitium (claims 43, 51, 58, and 65); and treat a patient having renal fibrosis by administering to the patient an antibody to Collagen XIII, wherein the antibody prevents the binding of Collagen XIII to α1β1 integrin positive monocytes (claims 44, 6, 52, 59, 66, and 71).

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

4-11-07
Date

Dominic Cosgrove

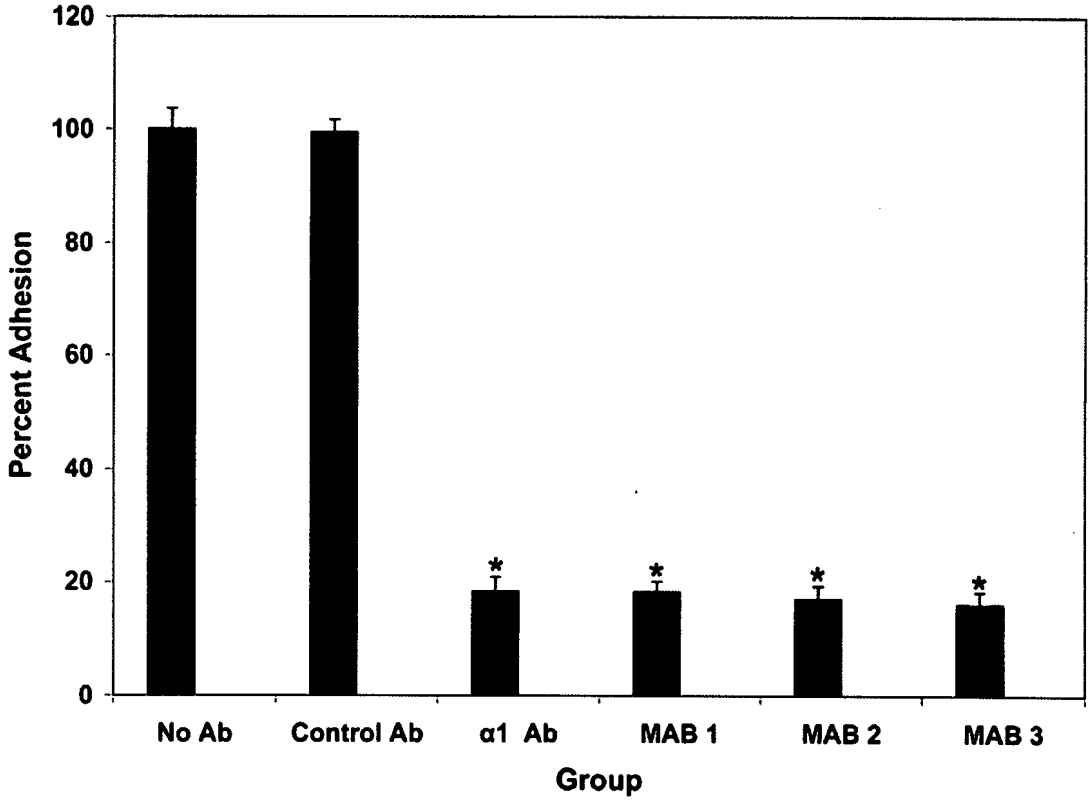

Exhibit A

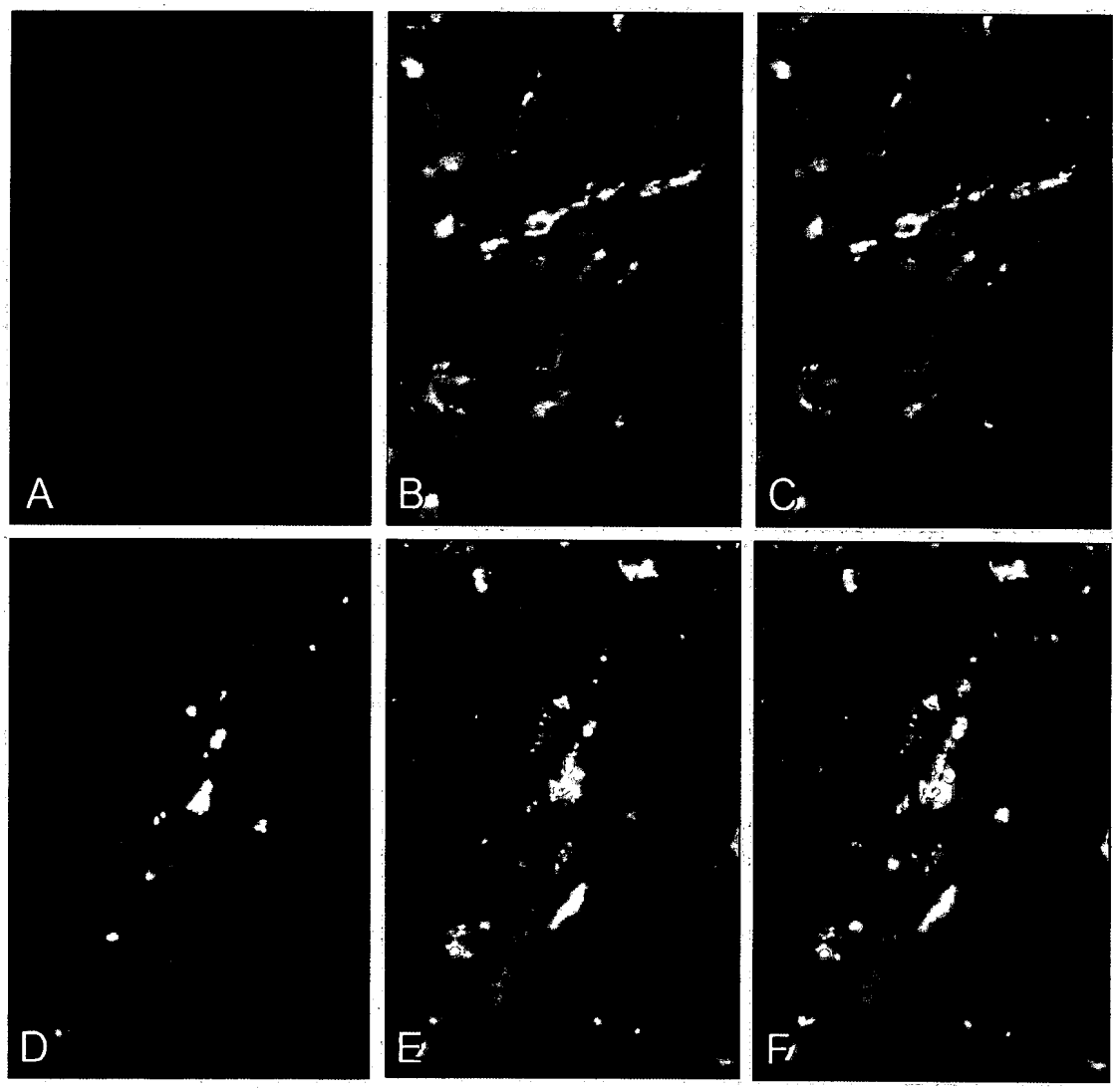

Exhibit B

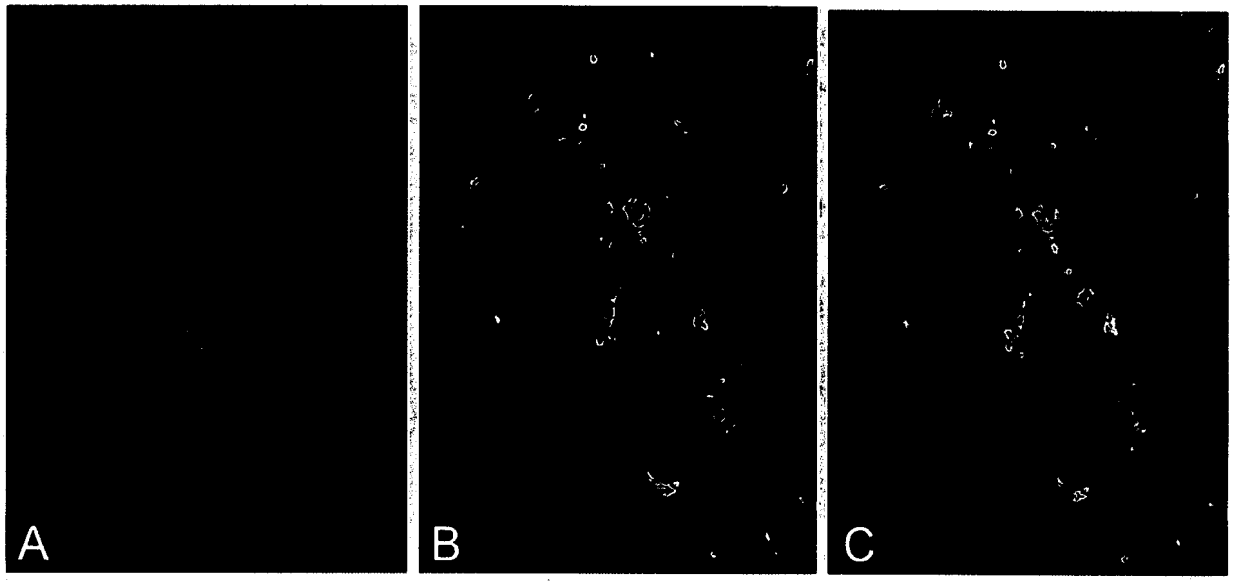

Exhibit C

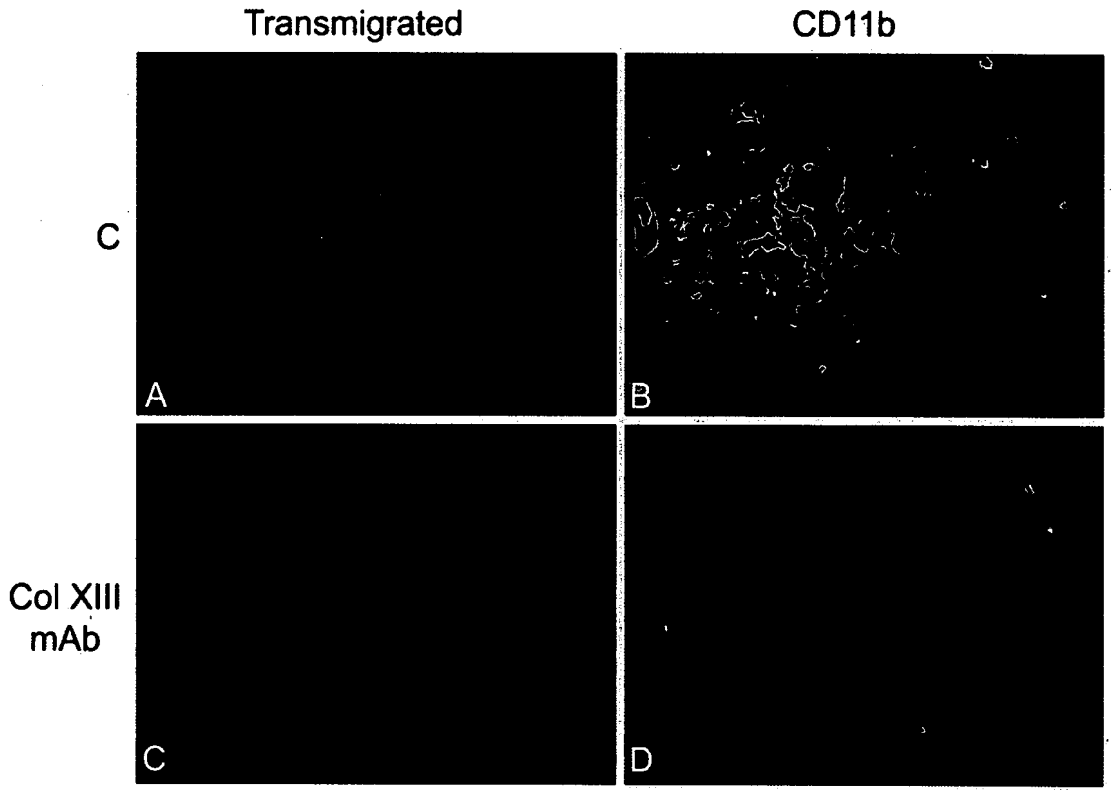

Exhibit D

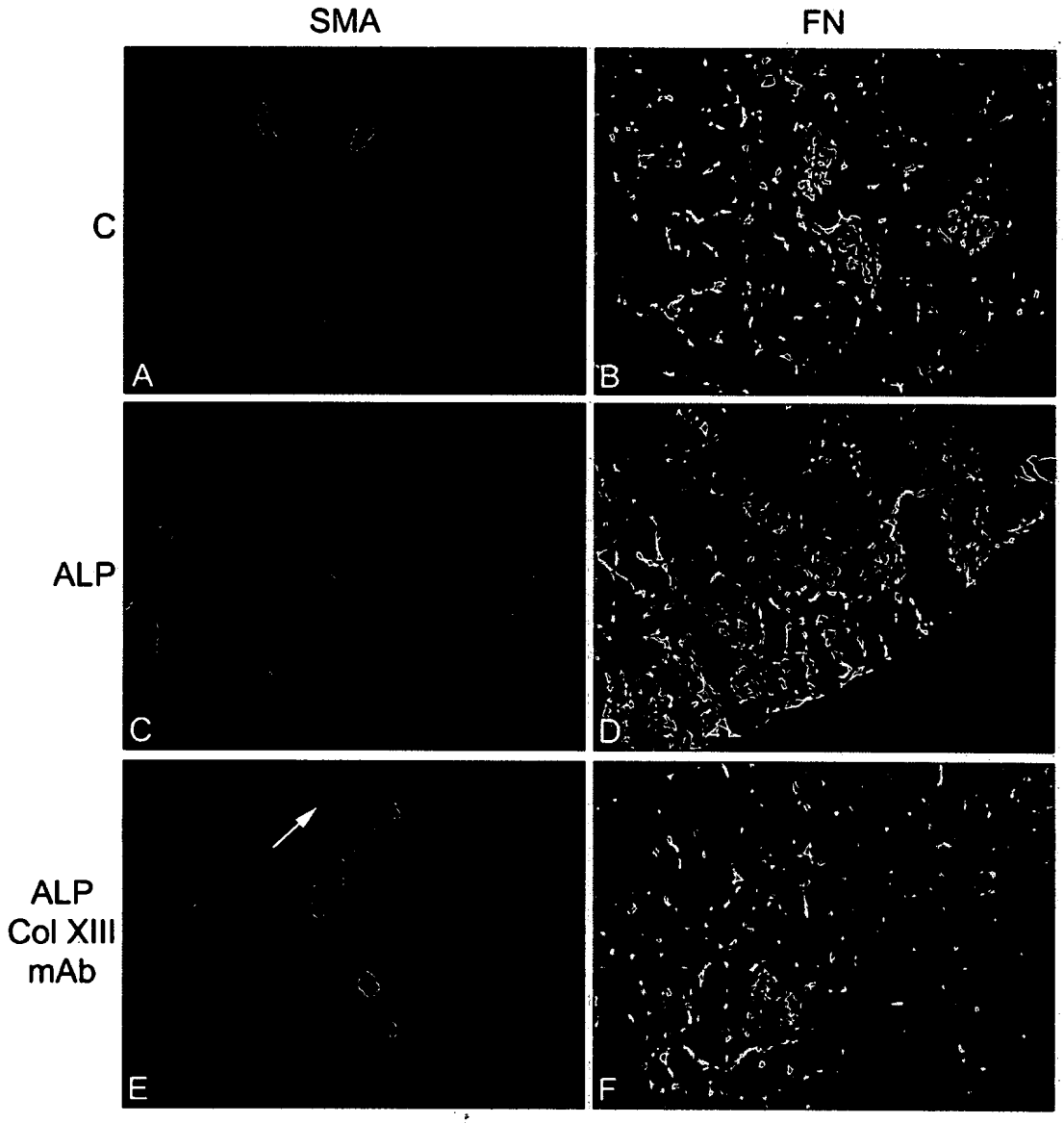

Exhibit E